

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



B F

(11)

EP 0 980 354 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:

19.06.2002 Bulletin 2002/25

(51) Int Cl.7: **C07C 401/00**

(86) International application number:

PCT/EP98/02390

(21) Application number: **98924177.3**

(87) International publication number:

WO 98/49138 (05.11.1998 Gazette 1998/44)

(22) Date of filing: **22.04.1998**

(54) **VITAMIN D3 ANALOGS WITH BIS C-20 SIDE CHAINS**

VITAMIN D3 ANALOGA MIT BIS-C-20-SEITENKETTEN

ANALOGUES DE LA VITAMINE D3 AVEC DES CHAINES LATERALES BIS C-20

(84) Designated Contracting States:

**AT BE CH DE DK ES FI FR GB GR IE IT LI LU NL
PT SE**

• **USKOKOVIC, Milan, Radoje**

Upper Montclair, NJ 07043 (US)

(30) Priority: **28.04.1997 US 44661 P**
22.01.1998 US 72140 P

(74) Representative: **Kjellsaa-Berger, Hanny, Dr. et al**

Grenzacherstrasse 124,

Postfach 3255

4070 Basel (CH)

(43) Date of publication of application:
23.02.2000 Bulletin 2000/08

(56) References cited:

US-A- 5 145 846

(73) Proprietor: **F. HOFFMANN-LA ROCHE AG**
4002 Basel (CH)

• **T. SUDA ET AL: BIOCHEMISTRY, vol. 9, no. 14,**
1970, pages 2917-2922, XP002081663

(72) Inventors:

• **MANCHAND, Percy, Sarwood**
Montclair, NJ 07042 (US)

• **A. KUREK-TYRLIK ET AL: LIEBIGS ANN./RECL.,**
no. 9, 1997, pages 1997-2001, XP002081664

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 980 354 B1

Description

[0001] This invention relates to Vitamin D₃ analogs, intermediates and methods for preparation of these analogs, pharmaceutical compositions comprising the analogs and the use of such analogs for the treatment of osteoporosis, primary and secondary hyperparathyroidism, autoimmune diseases and for the treatment and prevention of neoplastic diseases.

[0002] Osteoporosis is the most common form of metabolic bone disease and may be considered the symptomatic, fracture stage of bone loss (osteopenia). Although osteoporosis may occur secondary to a number of underlying diseases, 90% of all cases appear to be idiopathic. Postmenopausal women are at risk for idiopathic osteoporosis (postmenopausal or Type I osteoporosis); another particularly high risk group for idiopathic osteoporosis is the elderly of either sex (senile or Type II osteoporosis). Osteoporosis has also been related to corticosteroid use, immobilization or extended bed rest, alcoholism, diabetes, gonadotoxic chemo-therapy, hyperprolactinemia, anorexia nervosa, primary and secondary amenor-rhea, transplant immunosuppression, and oophorectomy. Postmenopausal osteoporosis is characterized by fractures of the spine, while femoral neck fractures are the dominant features of senile osteoporosis.

[0003] The mechanism by which bone is lost in osteoporotics is believed to involve an imbalance in the process by which the skeleton renews itself. This process has been termed bone remodeling. It occurs in a series of discrete pockets of activity. These pockets appear spontaneously within the bone matrix on a given bone surface as a site of bone resorption. Osteoclasts (bone dissolving or resorbing cells) are responsible for the resorption of a portion of bone of generally constant dimension. This resorption process is followed by the appearance of osteoblasts (bone forming cells) which then refill with new bone the cavity left by the osteoclasts.

[0004] In a healthy adult subject, osteoclasts and osteoblasts function so that bone formation and bone resorption are in balance. However, in osteoporotics an imbalance in the bone remodeling process develops which results in bone being replaced at a slower rate than it is being lost. Although this imbalance occurs to some extent in most individuals as they age, it is much more severe and occurs at a younger age in postmenopausal osteoporotics, following oophorectomy, or in iatrogenic situations such as those resulting from corticosteroid therapy or the immunosuppression practiced in organ transplantation.

[0005] Various approaches have been suggested for increasing bone mass in humans afflicted with osteoporosis, including administration of androgens, fluoride salts, and parathyroid hormone and modified versions of parathyroid hormone. It has also been suggested that bisphosphonates, calcitonin, calcium, 1,25-dihydroxy vitamin D₃ and some of its analogs, and/or estrogens, alone or in combination, may be useful for preserving existing bone mass.

[0006] Vitamin D₃ is a critical element in the metabolism of calcium, promoting intestinal absorption of calcium and phosphorus, maintaining adequate serum levels of calcium and phosphorus, and stimulating flux of calcium into and out of bone. Vitamin D₃ is hydroxylated *in vivo*, with the resulting 1 α ,25-dihydroxy metabolite being the active material. Animal studies with 1,25-(OH)₂ vitamin D₃ have suggested bone anabolic activity. Aerssens et al. in *Calcif Tissue Int*, 55:443-450 (1994) reported upon the effect of 1 α -hydroxy Vitamin D₃ on bone strength and composition in growing rats with and without corticosteroid treatment. However, human usage is restricted to antiresorption due to the poor therapeutic ratio (hypercalciuria and hypercalcemia as well as nephrotoxicity).

[0007] Dechant and Goa, in "Calcitriol. A review of its use in the treatment of postmenopausal osteoporosis and its potential in corticosteroid-induced osteoporosis", *Drugs Aging* [NEW ZEALAND 5 (4): 300-17 (1994)], reported that 1,25-dihydroxyvitamin D₃ (calcitriol) has shown efficacy in the treatment of postmenopausal osteoporosis (and promise in corticosteroid-induced osteoporosis) based upon a clinical trial in 622 women with postmenopausal osteoporosis. Patients with mild to moderate disease (but not those with more severe disease) who received calcitriol (0.25 microgram twice daily) had a significant 3-fold lower rate of new vertebral fractures after 3 years of treatment compared with patients receiving elemental calcium 1000 mg/day. In patients commencing long term treatment with prednisone or prednisolone, calcitriol 0.5 to 1.0 micrograms/day plus calcium 1000 mg/day, administered with or without intranasal calcitonin 400 IU/day, prevented steroid-induced bone loss. Overall, calcitriol was well tolerated. At recommended dosages hypercalcaemia was infrequent and mild, generally responding to reductions in calcium intake and/or calcitriol dosage. However, the narrow therapeutic window of calcitriol required that its use be adequately supervised, with periodic monitoring of serum calcium and creatinine levels. This study clearly identifies the key limitation of calcitriol therapy as the close proximity of therapeutic and toxic doses.

[0008] Baggiolini et al. in European Patent Publication No. 580,968 disclose fluorinated vitamin D₃ analogs, including 1 α -fluoro-25-hydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol, useful for the treatment of hyperproliferative disorders of the skin, for the treatment of cancer and leukemia, and for the treatment of sebaceous gland diseases. United States Patent Application Serial No. 08/560,080 discloses and claims the use of this compound for the restoration of bone mass and/or density in osteoporosis. Pending United States Patent Application, Serial No. 60/018,219 and United States Patent Application, "Fluorinated Vitamin D₃ analogs," filed March 19, 1997 also discloses the use of certain vitamin D₃ analogs for the treatment of osteoporosis.

[0009] Secondary hyperparathyroidism is a common finding in patients with chronic renal failure. It is established

[0012] The present invention provides Vitamin D₃ analogs of the Formula I



B₂ is a single bond, an E-double bond, a Z-double bond or a triple bond.

[0015] As used herein, the term "(C₁-C₄) alkyl" means a linear fully-saturated hydrocarbon radical having one to four carbon atoms or a branched fully saturated hydrocarbon radical having three to four carbon atoms; a "(C₁-C₄) fluoro-

alkyl" is an alkyl radical, as defined above, in which one or more hydrogen atoms attached to the carbon backbone have been substituted with one or more fluorine atoms.

[0016] As used herein, the term "(C₃-C₆) cycloalkyl" means a fully saturated cyclic hydrocarbon radical of three to six ring carbon atoms, e.g., cyclopropyl, cyclopentyl and the like; the term "(C₃-C₆) cyclofluoroalkyl" is a cycloalkyl radical, as defined above, in which one or more hydrogen atoms attached to the carbon backbone have been substituted with one or more fluorine atoms.

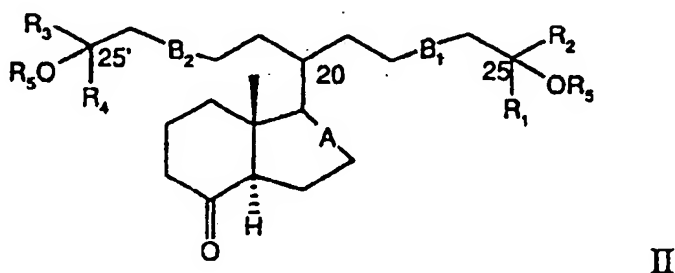
[0017] As used herein, the term "E" denotes a stereochemical configuration about a carbon-carbon double bond such that the two hydrogen atoms are attached to different carbon atoms and are on opposite sides of the carbon-carbon double bond; the term "Z" denotes a stereochemical configuration about a carbon-carbon double bond such that the two hydrogen atoms are attached to different carbon atoms and are on the same side of the carbon-carbon double bond.

[0018] "Pro-drugs" means any compound which releases an active parent drug according to Formula (I) *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs of a compound of Formula (I) are prepared by modifying functional groups present in the compound of Formula (I) in such a way that the modifications may be cleaved *in vivo* to release the parent compound. Prodrugs include compounds of Formula (I) wherein a hydroxy group in compound (I) is bonded to any group that may be cleaved *in vivo* to regenerate the free hydroxyl group. Examples of prodrugs include, but are not limited to esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylaminocarbonyl) and ethers of hydroxy functional groups in compounds of Formula (I), and the like. Such compounds are routinely made by one of skill in the art by acylating or etherifying the hydroxy group in the parent molecule.

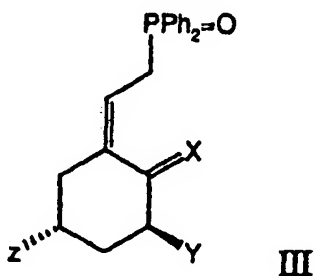
[0019] A "therapeutically effective amount" means the amount of a compound that, when administered to a mammal for treating or preventing a disease, is sufficient to effect such treatment or prevention for the disease. The "therapeutically effective amount" will vary depending on the compound, the disease and its severity and the age, weight, etc., of the mammal to be treated.

[0020] Compounds of this invention may generally be prepared by reaction and combination of fragments of Vitamin D₃ molecules. In this regard, the synthetic processes described in Shiuey et al., *J. Org. Chem.*, 55:243 (1990) may be used to prepare and combine the Vitamin D₃ fragments. The preparation of compounds of Formula I and intermediates in its preparation is illustrated by the following Reaction Schemes.

[0021] Compounds of Formula I are prepared from compounds of Formula II,



where R₁, R₂, R₃, R₄, A, B₁ and B₂ are described above, and R₅ is hydrogen or trimethylsilyl, by reaction with compounds of Formula III,



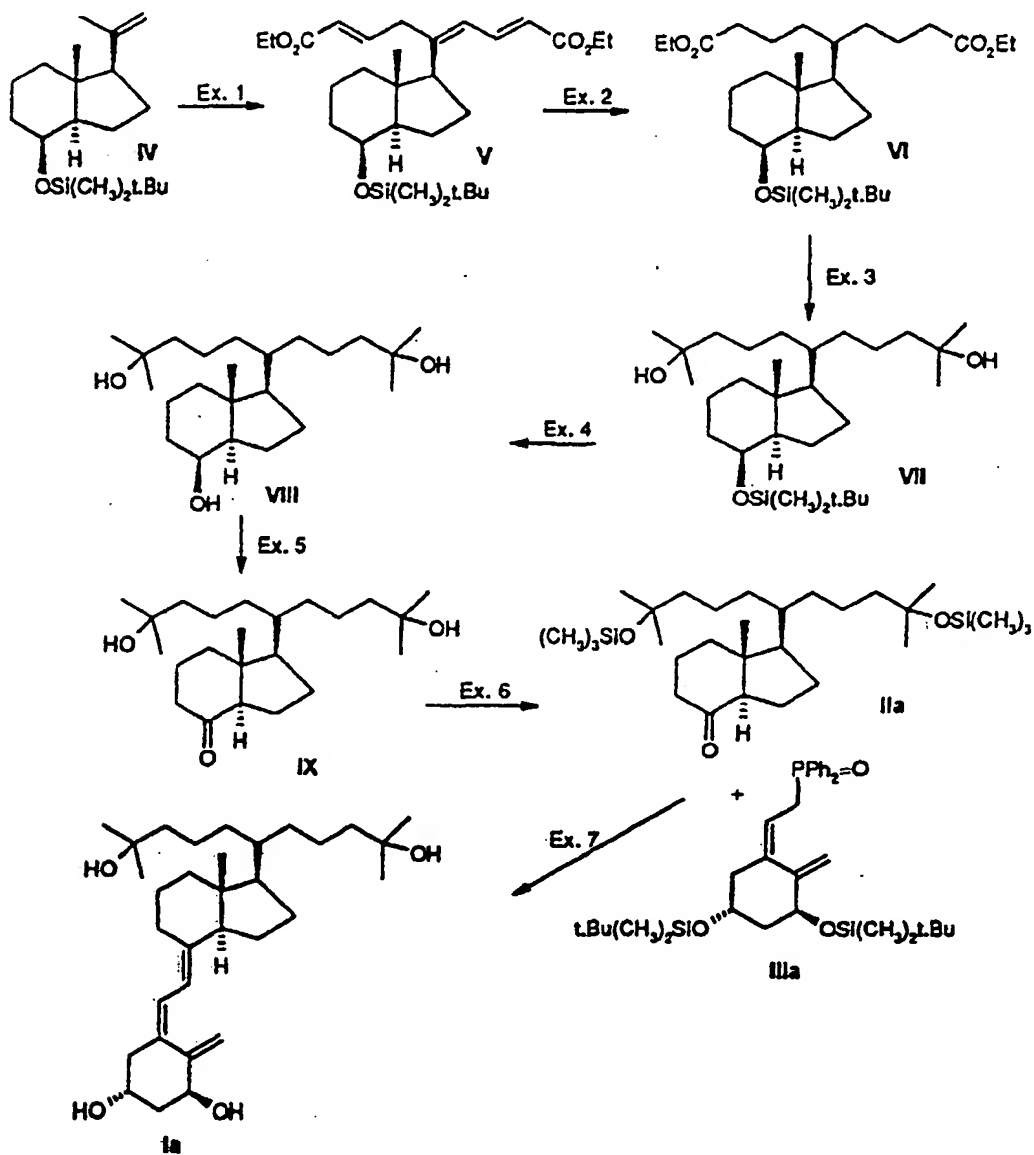
where X is described above, Y is hydrogen, fluorine or tert-butyl-dimethylsilyloxy group, and Z is a tertiary butyl-dimethylsilyloxy group, followed by removal of the silyl protecting groups.

[0022] In general, a compound of Formula III is reacted with n-butyllithium and a compound of Formula II in a mixture of hexane and tetrahydrofuran at a temperature of -78°C to give a compound of Formula I after removal of silyl protecting groups with tetrabutylammonium fluoride in tetrahydrofuran solvent.

[0023] It should be noted that although the shown intermediates have hydroxy groups typically protected as silyl ethers, the scope of the invention includes the use of alternative hydroxyl protecting groups known in the art as described in T.W. Greene, "Protective Groups in Organic Synthesis," Wiley, New York (1991) and J.F. McOmie, "Protective Groups in Organic Chemistry," Plenum Press, London (1973) together with alternative methods for deprotection.

[0024] Synthesis and purification of compounds of Formula III are known and conventional in this art. See, for example, U.S. Patent Nos., 5,086,191 and 5,616,759 to DeLuca et al., 5,087,619 to Baggiolini et al., 5,384,314 to Doran et al., 5,428,029 to Doran et al., 5,451,574 to Baggiolini et al.; European patent publication EP 0 808,832 A2, patent publication WO 96/31216 to Brasitus et al.; Shiuey et al., *J. Org. Chem.*, 55:243-247 (1990), Kiegel, J. et al. and *Tetr. Lett.*, 32:6057-6060 (1991), Perlman, K. L., et al., *Tetr. Lett.*, 32:7663-7666 (1991).

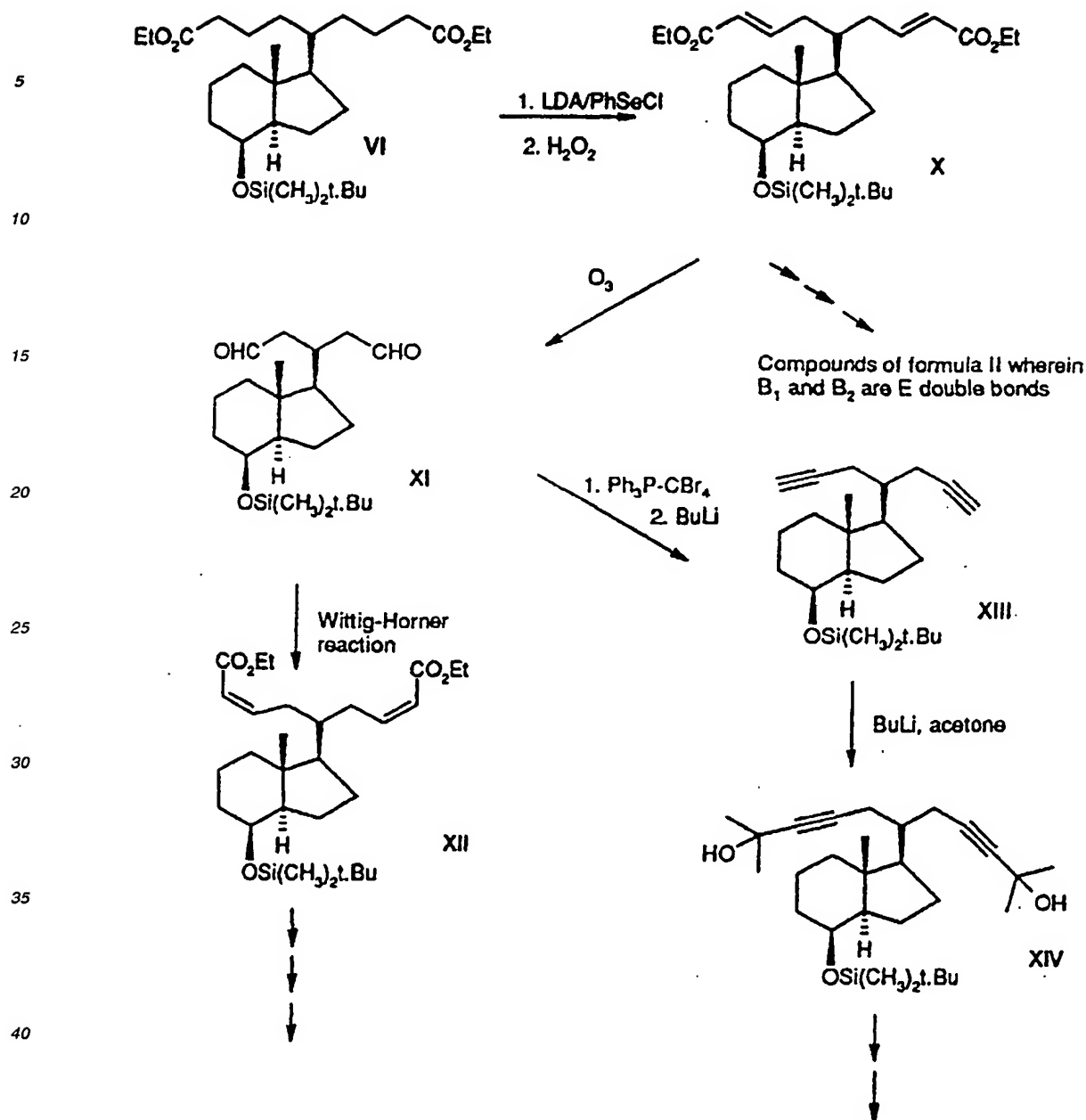
[0025] Reaction Scheme 1 shows a procedure for preparing a compound of Formula Ia where B_1 and B_2 are single bonds; R_1 - R_4 are each CH_3 ; X is CH_2 ; and Y and Z are OH.



[0026] In Reaction Scheme 1, the compound of Formula IV is a known compound prepared by dehydration of the corresponding precursor alcohol. See, for example, Wovkulich, P.M. et al., *Tetrahedron*, 40:2283 (1984). The compound

of Formula IV is converted to the compound of Formula V by reaction with ethyl propiolate in the presence of a Lewis acid, such as ethylaluminum dichloride. The reaction is conducted in a chlorinated hydrocarbon solvent such as dichloromethane, at room temperature. The compound of Formula V is converted to the compound of Formula VI by hydrogenation in the presence of a catalyst such as 10% palladium on carbon. The reaction is conducted in an ester solvent such as ethyl acetate, at one atmosphere pressure, at room temperature. The compound of the Formula VI is converted to the compound of Formula VII by reaction with methylmagnesium bromide in an ether solvent such as mixture of diethylether and tetrahydrofuran, at room temperature. The compound of Formula VII is converted to the compound of the Formula VIII by reaction with aqueous 30% fluorosilicic acid, in a mixture of acetonitrile and tetrahydrofuran as solvent, at room temperature. The compound of Formula VIII is oxidized to the compound of Formula IX with pyridinium dichromate in a chlorinated hydrocarbon solvent, such as dichloromethane, at room temperature. The compound of Formula IX is reacted with trimethyl-silylimidazole in dichloromethane as a solvent to give the compound of Formula IIa. The compound of Formula IIIa is reacted with n-butyllithium and the compound of Formula IIa in a mixture of hexane and tetrahydrofuran at a temperature of -78° C to give the compound of Formula Ia after removal of silyl protecting groups with tetrabutylammonium fluoride in tetrahydrofuran solvent.

[0027] Compounds of Formula I where A is a single bond or a double bond and B₁ and B₂ are double and triple bonds are prepared by reacting the corresponding precursors analogous to Formula II with compounds of Formula III. The corresponding precursors analogous to Formula II are prepared by methods known to one of skill in the art. Exemplary synthetic routes are shown in Reaction Scheme 2.



[0028] Compounds of Formula II where B₁ and B₂ are trans double bonds (E) are prepared by converting the compound of Formula VI to the corresponding bis-unsaturated ester X by treatment with lithium diisopropylamide and phenylselenenylchloride followed by hydrogen peroxide oxidation and selenoxide elimination. This bis-unsaturated ester X is converted to the precursor analogous to IIa in which B₁ and B₂ are trans double bonds (E) by reactions as shown in Scheme 1.

[0029] Compounds of Formula II where B₁ and B₂ are cis double bonds (Z) may be obtained by oxidative cleavage of the double bond of compounds of Formula X with reagents such as ozone or osmium tetroxide/sodium metaperiodate to produce an aldehyde of Formula XI. The aldehyde XI is then condensed using the Still modification of the Wittig-Horner reaction with a phosphonate ylide (CF₃CH₂O)₂P(O)CH₂C(O)OEt to give a bis-unsaturated ester XII in which the double bond is stereospecifically cis (Z). This bis-unsaturated ester XII is converted to the precursor analogous to IIa in which B₁ and B₂ are cis double bonds (Z) by reactions as shown in Scheme 1.

[0030] Compounds of Formula II where B₁ and B₂ are triple bonds can be prepared by dehydrogenating the unsaturated esters of Formula X or XII to the corresponding triple bond containing ester followed by condensation with an organometallic reagent as shown in Reaction Scheme 1 to give a compound analogous to that of Formula IIa. Formation

of the triple bond may also be accomplished by bromination/dehydrobromination of the double bond in compounds of Formula X and XII. Alternatively, the aldehyde of Formula XI can be treated with butyllithium and triphenylphosphine/carbon tetrabromide using the Corey-Fuchs reaction to generate acetylide anion XIII which is condensed with a ketone to produce the corresponding acetylenic alcohol XIV analogous to VII.

[0031] Similarly, starting from the analog of Compound VI where A is a double bond, one can obtain the precursor analogous to IIa where A is a double bond.

[0032] Compounds of Formula II with varying alkyl and fluoroalkyl groups R_1 - R_4 may be prepared by condensing the appropriate organometallic reagent, e.g., an alkylolithium or Grignard reagent with a C-25 ester as in V, VI, X and XII. Alternatively, these groups may be introduced by condensing an acetylide anion derived from an intermediate such as XIII with an appropriate ketone or fluoroketone (e.g. hexafluoroacetone).

[0033] In certain preferred embodiments, B_1 and B_2 are double bonds or triple bonds.

[0034] In other preferred embodiments, A is a double bond.

[0035] In other preferred embodiments, A is a single bond.

[0036] Preferred embodiments also include those in which X is CH_2 and Y is fluoro or hydroxy.

[0037] Other preferred embodiments are those where R_1 - R_4 are alkyl or fluoroalkyl, preferably trifluoromethyl.

[0038] Another aspect of the invention includes prodrugs of a compound of Formula I.

[0039] A number of different substituent preferences have been given above and following any of these substituent preferences results in a compound of the invention that is more preferred than one in which the particular substituent preference is not followed. However, these substituent preferences are generally independent, although some preferences are mutually exclusive, and following more than one of these preferences may result in a more preferred compound than one in which fewer of the substituent preferences are followed.

[0040] The compounds of this invention are useful for the prevention and treatment of a variety of mammalian conditions manifested by loss of bone mass. In particular, the compounds of this invention are indicated for the prophylaxis and therapeutic treatment of osteoporosis and osteopenia in mammals without inducing hypercalciuria, hypercalcemia, or nephrotoxicity. As used herein, "hypercalciuria" is excessive calcium in the urine, in humans corresponding to an excretion of greater than 4 mg/kg/day. This often results in nephrolithiasis (renal calculi). "Hypercalcemia" is an excessive concentration of calcium in the serum; in humans (and rats) this corresponds to greater than about 10.5 mg/dl. "Intolerable hypercalcemia", usually occurring at serum calcium concentrations greater than about 12 mg/dl, is associated with emotional lability, confusion, delirium, psychosis, stupor, and coma.

[0041] The compounds of this invention are expected to be useful in the treatment of Type I (postmenopausal), Type II (senile), and Type III (iatrogenic) osteoporosis, including that associated with immunosuppressive drugs used in organ transplantation, as well in the treatment of osteodystrophy due to renal dialysis and hyperparathyroidism.

[0042] Compounds of this invention are also useful in treating diseases caused by elevated levels of parathyroid hormone. In one aspect, compounds of the invention are used in treating secondary hyperparathyroidism associated with renal failure and in particular with reversing or reducing the bone loss associated with renal insufficiency. Other aspects include the treatment of renal osteodystrophy associated with late stage secondary hyperparathyroidism. Other aspects include the treatment of primary hyperparathyroidism.

[0043] Compounds of Formula I are also useful in treating neoplastic diseases such as leukemia, colon cancer, breast cancer and prostate cancer.

[0044] Compounds of Formula I are also useful in treating immunosuppressive and autoimmune diseases. Such diseases include, but are not limited to, multiple sclerosis, systemic lupus erythematosus, diabetes, thyroiditis and allograft rejection. In particular, compounds of Formula I are useful to treat diseases via modulation of the activity of the vitamin D_3 receptor (VDR). The utility of these compounds is demonstrated *in vivo* using murine models for these diseases as is well known in the art. See, e.g., Lemire et al., *Autoimmunity*, 12:143-148 (1992); Lemire et al., *J. Clin. Invest.*, 87:1103-1107 (1991), Lemire et al., *Endocrinology*, 135:2818 (1994), and Lemire et al., *J. Cellular Biochem.*, 49:26-31 (1992).

[0045] Generally, compounds of this invention do not cause the elevated calcium levels observed with other vitamin D_3 analogs such as $1,25(OH)_2$ vitamin D_3 , thus providing an improved therapeutic ratio and better treatment of the above diseases.

[0046] In general, the compound of this invention may be administered in amounts between about 0.0002 and 0.5 mg compound/kg body weight per day, preferably from about 0.001 to about 0.1 mg/kg body weight per day, most preferably from about 0.002 to about 0.02 mg/kg body weight per day. For a 50 kg human subject, the daily dose of active ingredient may be from about 0.01 to about 25 μ g, preferably from about 0.05 to about 10 μ g, most preferably from about 1.0 μ g to about 10 μ g per day. This dosage may be delivered in a conventional pharmaceutical composition by a single administration, by multiple applications, or via controlled release, as needed to achieve the most effective results, preferably once or twice daily by mouth. In certain situations, alternate day dosing may prove adequate to achieve the desired therapeutic response.

[0047] The selection of the exact dose and composition and the most appropriate delivery regimen will be influenced

by, *inter alia*, the pharmacological properties of the formulation, the nature and severity of the condition being treated, and the physical condition and mental acuity of the recipient. In the treatment of corticosteroid induced osteopenia, it is expected that the requisite dose will be greater for higher doses of corticosteroids.

[0048] Representative delivery regimens include oral, parenteral (including subcutaneous, intramuscular and intravenous), rectal, buccal (including sublingual), pulmonary, transdermal, and intranasal, most preferably oral. Administration may be continuous or intermittent (e.g., by bolus injection).

[0049] A further aspect of the present invention relates to pharmaceutical compositions comprising as an active ingredient a compound of the present invention, in admixture with a pharmaceutically acceptable, non-toxic carrier. As mentioned above, such compositions may be prepared for parenteral (subcutaneous, intramuscular or intravenous) administration, particularly in the form of liquid solutions or suspensions; for oral or buccal administration, particularly in the form of tablets or capsules; for pulmonary or intranasal administration, particularly in the form of powders, nasal drops or aerosols; and for rectal or transdermal administration.

[0050] The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA., (1985). Formulations for parenteral administration may contain as excipients sterile water or saline, alkylene glycols such as propylene glycol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Formulations for nasal administration may be solid and may contain excipients, for example, lactose or dextran, or may be aqueous or oily solutions for use in the form of nasal drops or metered spray. For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like.

[0051] Orally administrable compositions may comprise one or more physiologically compatible carriers and/or excipients and may be in solid or liquid form. Tablets and capsules may be prepared with binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or poly-vinylpyrrolidone; fillers, such as lactose, sucrose, corn starch, calcium phosphate, sorbitol, or glycine; lubricants, such as magnesium stearate, talc, polyethylene glycol, or silica; and surfactants, such as sodium lauryl sulfate. Liquid compositions may contain conventional additives such as suspending agents, for example sorbitol syrup, methyl cellulose; sugar syrup, gelatin, carboxymethylcellulose, or edible fats; emulsifying agents such as lecithin, or acacia; vegetable oils such as almond oil, coconut oil, cod liver oil, or peanut oil; preservatives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Liquid compositions may be encapsulated in, for example, gelatin to provide a unit dosage form.

[0052] Preferred solid oral dosage forms include tablets, two-piece hard shell capsules and soft elastic gelatin (SEG) capsules. SEG capsules are of particular interest because they provide distinct advantages over the other two forms (see Seager, H., "Soft gelatin capsules: a solution to many tableting problems"; *Pharmaceutical Technology*, 9, (1985). Some of the advantages of using SEG capsules are: a) dose-content uniformity is optimized in SEG capsules because the drug is dissolved or dispersed in a liquid that can be dosed into the capsules accurately b) drugs formulated as SEG capsules show good bioavailability because the drug is dissolved, solubilized or dispersed in an aqueous-miscible or oily liquid and therefore when released in the body the solutions dissolve or are emulsified to produce drug dispersions of high surface area and c) degradation of drugs that are sensitive to oxidation during long-term storage is prevented because the dry shell of soft gelatin provides a barrier against the diffusion of oxygen.

[0053] The dry shell formulation typically comprises of about 40% to 60% concentration of gelatin, about a 20% to 30% concentration of plasticizer (such as glycerin, sorbitol or propylene glycol) and about a 30 to 40% concentration of water. Other materials such as preservatives, dyes, opacifiers and flavours also may be present. The liquid fill material comprises a solid drug that has been dissolved, solubilized or dispersed (with suspending agents such as beeswax, hydrogenated castor oil or polyethylene glycol 4000) or a liquid drug in vehicles or combinations of vehicles such as mineral oil, vegetable oils, triglycerides, glycols, polyols and surface-active agents.

[0054] The following examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

EXPERIMENTAL

EXAMPLE 1

[1R-(1 α (2E,4E,7E),3 α β ,4 α ,7 α)]-5-[4-[[[(1,1-dimethylethyl)dimethyl silyl]oxy] Octahydro-7 α -methyl-1H-inden-1-yl]-2,4,7-nonatrienedioic acid diethyl ester (V).

[0055] To a stirred solution of 3.08 g (10.0 mmol) of [1R-(1 α ,3 α β ,4 α ,7 α)]-(1,1-dimethylethyl)dimethyl[[octahydro-7 α -methyl-1-(1-methylethenyl)-1H-inden-4-yl] oxy]silane and 3.92 g (40.0 mmol) of ethyl propiolate in 20 mL of dichloromethane was added 40 mL (40.0 mmol) of a 1.0 M solution of ethylaluminum dichloride in hexanes. The mixture was

stirred under argon at room temperature for 24 hrs, treated with 981 mg (10 mmol) of ethyl propiolate and 7.5 mL (7.5 mmol) of a 1.0 M solution of ethylaluminum dichloride in hexanes and stirred for an additional 18 hrs. The resultant orange-red solution was added portion-wise to a mixture of 200 mL ethyl acetate and 100 mL of 50% brine, and, after the fizzing had subsided, the organic phase was collected and the aqueous phase was re-extracted with 3 x 100 mL of ethyl acetate. The combined organic extracts were washed with 2 x 100 mL of 50% brine, dried (Na_2SO_4), and evaporated to give 5.76 g of a reddish gum, which was subjected to flash chromatography on 120 g of silica gel (40-65 μm mesh, 3.5 cm diameter column) with 10% ethyl acetate in hexanes as eluent, collecting 20-mL fractions. Fractions 21-32 were combined and evaporated to give 2.18 g of crude product. Further purification was achieved by HPLC (15-30 μm mesh silica gel, 50 cm x 50 mm column, flow rate of 70 mL/min) with 7.5 % ethyl acetate in hexanes as eluent to give 1.62 g (32%) of the titled compound, R_T 25 minutes, as a pale yellow guru: $[\alpha]_D^{25} + 83.50^\circ$ (EtOH, $c = 0.98$); UV (MeOH) 284 ($\epsilon = 28,173$), 207 ($\epsilon = 16,884$) nm; IR (CHCl_3) 1708, 1651, 1628 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.006 (6 H, s), 0.80 (3 H, s), 0.88 (9 H, s), 1.16 (1 H, t, $J = 7.6$ Hz), 1.28 (6 H, overlapping t, $J = 7$ Hz), 1.67 - 1.78, (6 H, m), 2.16 (1 H, t, $J = 9$ Hz), 3.00 (1 H, dd, $J = 6, 16$, Hz), 3.35 (1 H, dd, $J = 16, 4$ Hz), 4.02 (1 H, s), 4.16 (4 H, overlapping q, $J = 7$ Hz), 5.75 (1 H, d, $J = 16$ Hz), 5.84 (1 H, d, $J = 15$ Hz), 6.17 (1 H, d, $J = 11$ Hz), 6.88 (1 H, dt, $J = 16, 6$ Hz), 7.50 (1 H, dd, $J = 11, 15$, Hz); MS (EI) m/z 504 (M^+ , 23).

Anal. Calcd for $\text{C}_{29}\text{H}_{48}\text{O}_5\text{Si}$: C, 69.00; H, 9.58; Si, 5.56. Found: C, 68.94; H, 9.69; Si, 5.67.

EXAMPLE 2

[1R-(1 α ,3 $\alpha\beta$,4 α ,7 $\alpha\alpha$)]-5-[4-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]Octahydro-7a-methyl-1H-inden-1-yl]nonanedioic acid diethyl ester (VI).

[0056] A stirred solution of 1.009 g (2.0 mmol) of [1R-[1 α (2E,4E,7E),3 $\alpha\beta$,4 α ,7 $\alpha\alpha$]]-5-[4-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]octahydro-7a-methyl-1H-inden-1-yl]-2,4,7-nonatrienedioic acid diethyl ester in 50 mL of ethyl acetate was hydrogenated over 200 mg of 10% palladium on charcoal at room temperature and atmospheric pressure until hydrogen absorption ceased (140 mL of hydrogen was absorbed during 2.5 hrs). The mixture was filtered over a pad of Celite, which was washed with 4 x 50 mL of ethyl acetate, and the combined filtrate and washings were evaporated to give 1.07 g of a colorless oil. This was purified by flash chromatography on 60 g of silica gel (40-65 μm mesh, 3.5 cm diameter column) with 12% ethyl acetate in hexanes as eluent, collecting 20-mL fractions. Fractions 7-12 were combined and evaporated to give 964 mg (94%) of the titled compound as a colorless oil: $[\alpha]_D^{25} + 32.1^\circ$ (CHCl_3 , $c = 1.04$); IR (CHCl_3) 1726 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.00 (3 H, s), 0.01 (3 H, s), 0.87 (9 H, s), 0.88 (3 H, s), 1.27 (6 H, t, $J = 7$ Hz), 1.28 - 1.90 (21 H, m), 2.25 (4 H, br t), 3.98 (1 H, s), 4.11 (4 H, q, $J = 7$ Hz); MS (FAB) m/z 511 ($M^+ + 1$, 100). Anal. Calcd for $\text{C}_{29}\text{H}_{54}\text{O}_5\text{Si}$: C, 68.11; H, 10.66; Si, 5.50. Found: C, 68.21; H, 10.85; Si, 5.43.

EXAMPLE 3

[1R-(1 α ,3 $\alpha\beta$,4 α ,7 $\alpha\alpha$)]-6-[4-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]Octahydro-7a-methyl-1H-inden-1-yl]-2,10-dimethyl-2,10-undecanediol (VII).

[0057] To a stirred solution of 868 mg (1.7 mmol) of [1R-(1 α ,3 $\alpha\beta$,4 α ,7 $\alpha\alpha$)]-5-[4-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]octahydro-7a-methyl-1H-inden-1-yl]nonanedioic acid diethyl ester in 12 mL of anhydrous THF was added dropwise, with cooling (ice bath), 5.0 mL (15 mmol) of a 3.0 M solution of methylmagnesium bromide in ether. The mixture was stirred at room temperature for 45 minutes, cooled to 5 $^\circ\text{C}$, and quenched by the dropwise addition of 3.0 mL of saturated NH_4Cl . After the fizzing had subsided, 15 mL of ethyl acetate and 15 mL of saturated NH_4Cl were added, stirring was continued for 20 minutes, and the mixture was poured into 100 mL of ethyl acetate and 50 mL of saturated NH_4Cl . The organic phase was collected and the aqueous phase was re-extracted with 3 x 60 mL of ethyl acetate. The combined organic extracts were washed with 2 x 100 mL of 50% brine, dried (Na_2SO_4), and evaporated to give 814 mg of a colorless gum, which was purified by flash chromatography on 100 g of silica gel (40-65 μm mesh, 3.5 cm diameter column) with 50 % ethyl acetate in hexanes as eluent taking 20-mL fractions. Fractions 19-20 were combined and evaporated to give, after high vacuum drying (17 hrs), 763 mg (93%) of the titled compound as a colorless foam: $[\alpha]_D^{25} + 35.8^\circ$ (EtOH, $c = 1.02$); IR (CHCl_3) 3608 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.00 (6 H, s), 0.88 (9 H, s), 0.90 (3 H, s), 1.20 (12 H, s), 1.23 - 1.90 (27 H, m), 3.99 (1 H, s); MS (EI) m/z 482 (3, M^+). Anal. Calcd for $\text{C}_{29}\text{H}_{58}\text{O}_3\text{Si}$: C, 72.14; H, 12.11; Si, 5.82. Found: C, 72.18; H, 11.99; Si, 5.69.

EXAMPLE 4

[1S-(1 α ,3 $\alpha\beta$,4 α ,7 $\alpha\alpha$)]Octahydro-1-[5-hydroxy-1-(4-hydroxy-4-methylpentyl)-5-methylhexyl]-7a-methyl-4H-inden-4-ol. (VIII).

[0058] To a stirred solution of 700 mg (1.45 mmol) of [1R-(1 α ,3 $\alpha\beta$,4 α ,7 $\alpha\alpha$)]-6-[4-[(1,1-dimethylethyl)dimethylsilyl]oxy]octahydro-7a-methyl-1H-inden-1-yl]-2,10-dimethyl-2,10-undecanediol in 5 mL of THF and 15 mL of CH₃CN contained in a Teflon bottle was added 3.0 mL of an approximately 30 % aqueous solution of fluorosilicic acid (prepared according to A. S. Pilcher and P. DeShong, *J. Org. Chem.*, 1993, 58, 5130) and the mixture was stirred under argon at room temperature for 1.0 h. Four 2.0-mL portions of the fluorosilicic acid solution were then added at hourly intervals, for a total of 11 mL of reagent and a reaction time of 5 hrs. The reaction mixture was poured cautiously into a mixture of 125 mL of ethyl acetate and 75 mL of saturated aqueous KHCO₃ solution. After the fizzing had subsided, the organic phase was collected and the aqueous phase was re-extracted with 3 x 75 mL of ethyl acetate. The organic extracts were washed with 125 mL of 50% brine, dried (Na₂SO₄), and evaporated to give 534 mg of a gum, which was purified by flash chromatography on 70 g of silica gel (40-65 μ m mesh, 3.5 cm diameter column) with 70% ethyl acetate as eluent, taking 20-mL fractions. Fractions 17-30 were combined, filtered and evaporated, and the residue was kept under high vacuum for 4 hrs to give 458 mg (85%) of the titled compound as a colorless foam: $[\alpha]_D^{25}$ +26.2° (CHCl₃, c = 0.76); IR (CHCl₃) 3608 cm⁻¹; ¹H NMR (CDCl₃) δ 0.93 (3 H, s), 1.21 (12 H, s), 1.24 - 1.60 (24 H, m), 1.79-1.95 (4 H, m), 4.07 (1H,s); MS (FAB) m/z 369 (M⁺+H).

EXAMPLE 5

[1S-(1 α ,3 $\alpha\beta$,7 $\alpha\alpha$)]Octahydro-1-[5-hydroxy-1-(4-hydroxy-4-methylpentyl)-5-methylhexyl]-7a-methyl-4H-inden-4-one (IX).

[0059] To a stirred solution of 400 mg (1.08 mmol) of [1S-(1 α ,3 $\alpha\beta$,4 α ,7 $\alpha\alpha$)]octahydro-1-[5-hydroxy-1-(4-hydroxy-4-methylpentyl)-5-methylhexyl]-7a-methyl-4H-inden-4-ol in 8.0 mL of dichloromethane was added 1.30 g (3.45 mmol) of pyridinium dichromate and the mixture was stirred at room temperature for 4.75 hrs. It was diluted with 20 mL of diisopropyl ether, stirred for a further 15 minutes and filtered over a pad of Celite. The Celite was washed with 4 x 40 mL of diisopropyl ether and the combined filtrate and washings were evaporated to give 405 mg of a pale yellow gum, which was purified by flash chromatography on 70-g of silica gel (40-65 μ m mesh, 3.5 cm diameter column) with 75% ethyl acetate in hexanes as eluent taking 20-mL fractions. Fractions 17-30 were combined and evaporated to give a colorless gum, which was kept under high vacuum for 4.5 hrs to give 372 mg (94%) of the titled compound as a colorless gum: $[\alpha]_D^{25}$ 0.45° (EtOH, c = 0.92); IR (CHCl₃) 3608,1706 cm⁻¹; ¹H NMR (CDCl₃) δ 0.63 (3 H, s), 1.22 (12 H, s), 1.30-2.10 (22 H, m), 2.20-2.28 (2 H, m), 2.45 (1 H; dd, J = 7.6,11 Hz); MS m/z 348 (M⁺-18).

EXAMPLE 6

[1S-(1 α ,3 $\alpha\beta$,7 $\alpha\alpha$)]Octahydro-7a-methyl-1-[5-methyl-1-[4-methyl-4-[(trimethylsilyl)oxy]pentyl]-5-[(trimethylsilyl)oxy]hexyl]-4H-inden-4-one (IIa).

[0060] To a stirred solution of 366.6 mg (1.0 mmol) of [1S-(1 α ,3 $\alpha\beta$,7 $\alpha\alpha$)]octahydro-1-[5-hydroxy-1-(4-hydroxy-4-methylpentyl)-5-methylhexyl]-7a-methyl-4H-inden-4-one in 10.0 mL of dichloromethane was added 1.25 mL (8.5 mmol) of 1-(trimethylsilyl)imidazole and the mixture was stirred under argon at room temperature for 4.25 hrs. It was diluted with 7.0 mL of water, stirred for a further 15 minutes, and poured into a mixture of 75 mL of ethyl acetate and 50 mL of 50% brine. The organic phase was collected and the aqueous phase was re-extracted with 3 x 50 mL of ethyl acetate. The combined organic extracts were washed with 3 x 75 mL of 50% brine, dried (Na₂SO₄), and evaporated to give a colorless oil, which was purified by flash chromatography on 65 g of silica gel (40-65 μ m mesh, 3.5 cm diameter column) with 20% ethyl acetate in hexanes as eluent, taking 20-mL fractions. Fractions 5-7 were combined, concentrated to ca. 5 mL, filtered through a 0.45 μ m filter (Millex-HV) and evaporated to give a colorless oil, which was kept under high vacuum for 18 hrs to give 469 mg (91%) of the titled compound: $[\alpha]_D^{25}$ -3.21° (CHCl₃, c = 0.87); IR (CHCl₃) 1706 cm⁻¹; ¹H NMR (CDCl₃) δ 0.01 (18 H, s), 0.63 (3 H, s), 1.20 (6 H, s), 1.21 (6 H, s), 1.26 - 1.49 (14 H, m), 1.50 - 2.10 (8 H, m), 2.21-2.31 (2 H, m), 2.46 (1 H, dd, J = 12,11 Hz); MS (EI) m/z 495 (M⁺-15).
Anal. Calcd for C₂₉H₅₈O₃Si₂: C, 68.17; H, 11.44; Si, 10.99. Found: C, 68.19; H, 11.41; Si, 11.07.

EXAMPLE 7

(1 α ,3 β ,5Z,7E)-21-(3-hydroxy-3-methylbutyl)-9,10-Secocholesta-5,7,10, (19)-triene-1,3,25-triol (Ia).

[0061] To a stirred, cold (-78 °C) solution of 466 mg (0.8 mmol) of the reagent [3S-(1Z,3 α ,5 β)]-[2-[3,5-bis[[[1,1-dimethylethyl]dimethylsilyl]oxy]-2-methylenecyclohexylidene]ethyl]di-phenylphosphine oxide in 4.0 mL of anhydrous THF was added 0.5 mL of a 1.6 M solution of n-butyllithium in hexanes. The resultant deep red solution was stirred at -78 °C for 7 minutes, treated with 204 mg (0.40 mmol) of [1S-(1 α ,3 α , β ,7 α)]octahydro-7a-methyl-1-[5-methyl-1-[4-methyl-4-[(trimethylsilyl)oxy]pentyl]-5-[(trimethylsilyl)oxy]hexyl]-4H-inden-4-one in 3.0 mL of anhydrous THF, and stirred at -78 °C for 3 hrs. The mixture was allowed to warm to room temperature, quenched with 5 mL of a 1:1 mixture of 2N Rochelle salt solution and 2N KHCO₃ solution, stirred for an additional 15 minutes, and poured into a mixture of 80 mL of ethyl acetate and 50 mL of 1:1 2N Rochelle salt solution and 2N KHCO₃ solution. The organic phase was collected and the aqueous phase was re-extracted with 3 x 60 mL of ethyl acetate. The combined organic extracts were washed with 3 x 75 mL of 50% brine, dried (Na₂SO₄) and evaporated to give 1.29 g of a gum, which was purified by flash chromatography on 60 g of silica gel (40-65 μ m mesh; 3.5 cm diameter column) with 8% ethyl acetate in hexanes, taking 20-mL fractions. Fractions 5 and 6 were combined and evaporated to give 208 mg of a colorless gum. The latter was dissolved in 4.0 mL of THF, treated with 4.0 mL of a 1.0M solution of tetrabutylammonium fluoride in THF, and the solution was stirred under argon for 17 hrs. It was diluted with 5.0 mL of water, stirred for an additional 15 minutes, and poured into a mixture of 80 mL of 80% ethyl acetate in hexanes and 50 mL of water. The organic phase was collected and the aqueous phase was re-extracted with 4 x 80 mL of 80% ethyl acetate in hexanes. The combined organic extracts were washed with 4 x 80 mL of 50% brine, dried (Na₂SO₄), and evaporated to give 139 mg of a semi-solid, which was purified by flash chromatography on 60 g of silica gel (40-65 μ m mesh; 3.5 cm diameter column) with 6% 2-propanol in ethyl acetate as eluent taking 20-mL fractions. Fractions 17-25 were combined and evaporated to give 108 mg of a colorless foam. This was further purified by HPLC (15-30 μ m mesh silica gel, 50 cm x 50 mm column; flow rate of 70 mL/min) with 3% 2-propanol in ethyl acetate as eluent. The material eluting with an RT of 35 minutes was collected and the solvents were evaporated to give a colorless semi-solid. This was dissolved in 15 mL of anhydrous methyl formate, filtered through a 0.45 μ m filter (Millex-HV), concentrated, and kept under high vacuum for 4 hrs to give 82 mg of the titled compound as a colorless, amorphous solid: [α]_D²⁵ +13.8° (EtOH, c = 0.5); UV (MeOH) 263 (ϵ = 17,545), 212 (ϵ = 14,702) nm; IR (CHCl₃) 3608 cm⁻¹; ¹H NMR (CDCl₃) δ 0.53 (3 H, s), 1.21 (12 H, s), 1.26-2.17 (32H, m), 2.30 (1 H, dd, J = 10,7 Hz), 2.59 (1 H, d, J = 11 Hz), 2.83 (1 H, d, J = 13 Hz), 5.00 (1 H, s), 5.33 (1 H, s), 6.02 (1 H, d, J = 11 Hz), 6.37 (1 H, d, J = 11 Hz); HRMS (EI): Calcd for C₃₂H₅₄O₂: m/z 502.4022. Found m/z 502.4024.

EXAMPLE 8.

21-(3-Hydroxy-3-methylbutyl)-1 α -fluoro-25-hydroxycholecalciferol.

[0062] To a stirred, cold (-78 °C) solution of 320 mg (0.67 mmol) of the reagent (S-trans-1-fluoro-5[[dimethyl (1,1-dimethylethyl)silyl]oxy]-2-methenyl-3 [diphenylphosphinyl]ethylidene]cyclohexane, in 4.0 mL of anhydrous THF was added 0.42 mL (0.67 mmol) of a 1.6 M solution of n-butyllithium in hexanes. The resultant deep red solution was stirred at -78 °C for 7 minutes, treated with 118 mg (0.23 mmol) of [1R-(1 α ,3 α , β ,7 α)]octahydro-7a-methyl-1-[5-methyl-1-[4-methyl-4-[(tri-methylsilyl)oxy]pentyl]-5-[(trimethylsilyl)oxy]hexyl]-4H-inden-4-one in 2.0 mL of anhydrous THF, and stirred at -78 °C for 4.5 hours. The mixture was allowed to warm to room temperature, stirred for 20 minutes, and quenched with 5 mL of a 1:1 mixture of 1N Rochelle salt solution and 1N KHCO₃ solution. After 15 minutes, the mixture was poured into 50 mL of a 1:1 mixture of 1N Rochelle salt solution and 1N KHCO₃ solution. The organic phase was separated and the aqueous phase was re-extracted with 3 x 40 mL of ethyl acetate. The combined organic extracts were washed with 100 mL of 10% brine, dried (Na₂SO₄), and evaporated to give 426 mg of a colorless gum; which was purified by flash chromatography on 40 g of silica gel (40-65 μ m mesh; 3.5 cm diameter column) with 5% ethyl acetate in hexanes as eluent, taking 15- mL fractions. Fractions 5-7 were combined and evaporated to give 144 mg of a colorless gum. The latter was dissolved in 3.0 mL of THF, treated with 2.0 mL of a 1.0 M solution of tetra-n-butylammonium fluoride in THF, and the solution was stirred under argon at room temperature for 17 hours. It was diluted with 5.0 mL of water, stirred for 10 minutes and poured into 50 mL of ethyl acetate and 40 mL of water. The organic phase was separated and the aqueous phase was re-extracted with 3 x 50 mL of ethyl acetate. The combined organic extracts were washed with 5 x 100 mL of water, dried (Na₂SO₄) and evaporated to give 78 mg of a gum, which was purified by flash chromatography on 40 g of silica gel (40-65 μ m mesh; 3.5 diameter column) with 90% ethyl acetate in hexanes as eluent, taking 15-mL fractions. Fractions 14-20 were combined and evaporated to give 57 mg of a colorless semi-solid, which was dissolved in 20 mL of anhydrous methyl formate and filtered through a 0.4 μ m filter. The filtrate was concentrated to 1.0 mL, kept at 0 °C for 1.0 hour, and the crystals were collected by filtration to give 42 mg of the title compound, mp 96-98 °C; [α]_D²⁵ +38.6° (MeOH, c = 0.5); UV (MeOH) 270 (ϵ = 14,136), 242 (ϵ =

14,350), 210 ($\epsilon = 13,564$) nm; IR (CHCl₃) 3610 cm⁻¹; ¹H NMR (CDCl₃) δ 0.54 (3 H, s), 1.21 (12 H, s), 1.22 - 1.60 (21 H, m), 1.69 (2 H, m), 1.80 (1 H, m), 2.0 (3 H, m), 2.17 (1 H, m), 2.31 (1 H, m), 2.62 (1 H, d, J = 12 Hz), 2.82 (1 H, d, J = 12 Hz), 4.22 (1 H, br s), 5.07 (1 H, br t), 5.10 (1 H, s), 5.19 (1 H, br t), 5.39 (1 H, s), 6.02 (1 H, d, J = 11 Hz), 6.40 (1 H, d, J = 11 Hz); MS (FAB) m/z 504.5 (M⁺, 60).

EXAMPLE 9

[0063] 21-(3-Hydroxy-3-methylbutyl)-1,25-dihydroxy-19-nor-cholecalciferol. To a stirred, cold (-78 °C) solution of 571 mg (1.0 mmol) of the reagent [3R-(3 α ,5 β ,Z)-3,5-bis[[1,1-dimethylethyl]dimethylsilyl]oxy]cyclohexylidene] ethyl]-diphenylphosphine oxide, in 6.0 mL of anhydrous THF was added 0.65 mL (1.04 mmol) of a 1.6 M solution of n-butyllithium in hexanes. The resultant deep red solution was stirred at -78 °C for 10 minutes, treated with 204.4 mg (0.4 mmol) of [1R-(1 α ,3 α ,7 α)]octahydro-7 α -methyl-1-[5-methyl-1-[4-methyl-4-[(trimethylsilyl)oxy]pentyl]-5-[(trimethylsilyl)oxy]hexyl]-4H-inden-4-one in 2.5 mL of anhydrous THF, and stirred at -78 °C for 3.0 hours. The mixture was allowed to warm to room temperature, stirred for 15 minutes and quenched with 15 mL of a 1:1 mixture of 1N Rochelle salt solution and 1N KHCO₃ solution. After 10 minutes, the mixture was poured into a mixture of 70 mL of ethyl acetate and 40 mL of a 1:1 mixture of 1N Rochelle salt solution and 1N KHCO₃ solution. The organic phase was separated and the aqueous phase was re-extracted with 3 x 70 mL of ethyl acetate. The combined organic extracts were washed with 100 mL of 10% brine, dried (Na₂SO₄), and evaporated to give 760 mg of a colorless gum, which was purified by flash chromatography on 60 grams of silica gel (40-65 μ m mesh; 3.5 cm diameter column) with 5% ethyl acetate in hexanes as eluent, taking 15 mL fractions. Fractions 5-10 were combined and evaporated to give 304 mg of a colorless gum. The latter was dissolved in 4.0 mL of THF, treated with 5.0 mL of a 1.0 M solution of tetra-n-butylammonium fluoride in THF, and the solution was stirred under argon at room temperature for 42 hours. It was diluted with 15 mL of water, stirred for 15 minutes, and poured into a mixture of 75 mL of ethyl acetate and 50 mL of 10% brine. The organic phase was separated and the aqueous phase was re-extracted with 3 x 70 mL of ethyl acetate. The combined organic extracts were washed with 5 x 100 mL of water, dried (Na₂SO₄) and evaporated to give 186 mg of a semi-solid, which was purified by flash chromatography on 50 g of silica gel (40-65 μ m mesh; 3.5 cm diameter column) with 7.5% 2-propanol in ethyl acetate as eluent, taking 15-mL fractions. Fractions 11-29 were combined and evaporated. The residue was dissolved in 20 mL of anhydrous methyl formate and the solution was filtered through a 0.4 μ m filter. Evaporation of the filtrate gave 154 mg of the title compound as a colorless solid: [a]_D²⁰ + 50.93° (MeOH, c = 0.32); ¹H NMR (CDCl₃) δ 0.54 (3 H, s), 1.21 (12 H, s), 1.2-2.0 (27 H, m), 2.20 (2 H, m), 2.48 (1 H, d, J = 12 Hz), 2.25 (2 H, m), 2.82 (1 H, s), 4.06 (1 H, br s), 4.10 (1 H, br s), 5.85 (1 H, d, J = 12 Hz), 6.30 (1 H, d, J = 12 Hz); MS (FAB) m/z 490.4 (M⁺, 30).

EXAMPLE 10

EFFECT OF VITAMIN D₃ ANALOGS ON SECONDARY HYPERPARATHYROIDISM IN THE RAT RENAL INSUFFICIENCY MODEL

[0064] The parathyroid hormone suppressive activity of the vitamin D₃ analogs of this invention was demonstrated in rats with secondary hyperparathyroidism due to renal failure using the 7/8 nephrectomy induced rat model of renal failure (*Kidney International*, M. Fukugawa et al., 39:874-881 (1991)).

Test Materials:

[0065]

Compound of Formula Ia
1,25(OH)₂ vitamin D₃ (control)
Vehicle - Miglyol 812

[0066] Female Sprague Dawley rats were anesthetized, their right kidney removed and 2-3 branches of the left renal artery were ligated to achieve 7/8 nephrectomy. They were placed on a high phosphorous diet (0.6% Ca and 0.8 phosphorous). Approximately 3-6 weeks after surgery, rats were bled to screen serum PTH levels and rats with PTH levels between 100-500 pg/ml were selected for the study. Rats were divided into five groups which were treated as shown in the Table below.

Group	Number of rats	Treatment
1	6	Formula Ia, 10 µg/kg/day, po
2	6	Formula Ia, 1 µg/kg/day, po
3	6	Formula Ia, 0.1 µg/kg/day, po
4	5	1,25(OH) ₂ vit. D ₃ , 0.2 µg/kg/day, po
5	5	Vehicle, po

[0067] There was a pre-bleed (T=0) and each group was dosed daily for seven days by oral gavage with either the compound of Formula Ia (10, 1 or 0.1 µg/kg/day), vehicle control or 1,24(OH)₂ vitamin D₃ positive control. Compounds were predissolved in ethanol and diluted with vehicle (Miglyol 812) followed by evaporation of the ethanol.

[0068] After the last day of dosing, the animals were bled again (T=1) and sacrificed. Serum PTH assays were done with Nichols Institute Diagnostic Kit #40-2240. Serum calcium assays were done with Sigma Diagnostic Kit #587 with o-cresolphthalein. Serum creatinine assays were done with Sigma Diagnostic Kit #1600-320 with ammonium molybdate.

Group	PTH pg/ml T=1-T=0	Final Ca Levels (mg/ml)
Formula Ia (10 µg/kg)	-132	11.97
Formula Ia (1 µg/kg)	-124	10.35
Formula Ia (0.1 µg/kg)	112	10.20
1,25(OH) ₂ vit. D ₃ (0.1 µg/kg)	-66	10.82
Vehicle	156	9.69

[0069] The results show that the compound of Formula Ia is more effective than 1,25(OH)₂ vit. D₃ in suppressing the elevated levels of parathyroid hormone without causing elevated calcium levels.

EXAMPLE 11

BONE ANABOLISM IN THE RAT

[0070] Three month old rats are ovariectomized (Ovx) and administered either 1,25-dihydroxy vitamin D₃ (vit. D in Table) or one of the compounds of the present invention once a day by mouth starting at 3 weeks post-ovariectomy and continuing until final sacrifice at 6 weeks post-ovariectomy. Control groups, both sham (rats that were not ovariectomized) and Ovx, received vehicle only. Blood and urine samples were collected twice, at 4 weeks post-ovariectomy and again at the 6 week mark and the amount of serum and urine calcium was determined. The final femoral calcium determined upon sacrifice 6 weeks post-ovariectomy.

[0071] The bone mineral density of the right femur was determined by using a High Resolution Software Package on a QDR-1000W Bone Densitometer™ (Hologic, Waltham, MA). The animals were scanned by placing them on a scanning block in a supine position such that the right leg was perpendicular to the main body and the tibia was perpendicular to the femur.

[0072] The compounds of the present invention are more effective than 1,25-dihydroxy vitamin D₃ at bone accretion and do not induce hypercalciuria, nephrotoxicity, or hypercalcemia at therapeutically effective doses.

EXAMPLE 12

AUTOIMMUNITY IN THE RAT EAE MODEL

[0073] The ability of vitamin D₃ analogs to treat autoimmune diseases is demonstrated *in vivo* in the rat model of experimental autoimmune encephalomyelitis (EAE).

[0074] Female Lewis rats are immunized with a 1:1 mixture of guinea pig spinal cord homogenate and modified Freund's complete adjuvant (4 mg/ml M. tuberculosis in incomplete Freund's adjuvant) via footpad injection. Compounds or vehicle is administered either subcutaneously or per os for 12 days starting on the 5th day after immunization. The animals are monitored for the onset of symptoms of EAE-loss of tail tonicity, weakness of hindlimbs, staggering

gait, paralysis etc.

[0075] The compounds of Formula 1 are effective in reducing the symptoms of EAE in the rat model.

EXAMPLE 13

CELL PROLIFERATION ASSAY IN MCF-7 BREAST CANCER CELLS

[0076] MCF-7 cells are human mammary carcinoma cells that are positive for estrogen receptors. The potential activity of vitamin D₃ analogs against breast cancer was assessed from inhibition of proliferation of MCF-7 cells in culture.

[0077] MCF-7 cells were plated at 5000 cells/well in 96-well plates and incubated at 37°C in 5% CO₂/95% air in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum 700 nM insulin, 2 mM glutamine, 0.1 mM MEM non-essential amino acids and 1 mM sodium pyruvate. Stock solutions of vitamin D₃ analogs were prepared at a concentration of 10 mM in absolute ethanol and stored at -20°C under argon. One day after plating, MCF-7 cells were refed with either control medium or medium containing varying concentrations of the vitamin D₃ analog. After a further 7 days of culture, the number of MCF-7 cells in each well was assessed from the reduction of the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), as described by F. Denizot and R. Lang, *J. Immunological Methods*, Vol. 89:271-277 (1986). MTT was added to each well to a final concentration of 1 mg/ml and the cells were incubated for a period of three hours, after which reduced MTT was extracted using 95% ethanol and the optical density was measured at a wavelength of 570 nm.

[0078] For each vitamin D₃ analog, the IC₅₀ value was determined from a graph relating the optical density at 570 nm to the concentration used. The IC₅₀ value was defined as the concentration of the vitamin D₃ analog corresponding to half-maximal reduction in 570 nm absorbance.

Vitamin D ₃ Analogs	IC ₅₀ nM
1,25-Dihydroxy-cholecalciferol	40
21-(3-Hydroxy-3-methyl)butyl-1,25-dihydroxy-cholecalciferol	0.8

[0079] The results of the above test show that 21-(3-Hydroxy-3-methyl)butyl-1,25-dihydroxy-cholecalciferol is approximately 50 times more potent than 1,25-dihydroxy-cholecalciferol in inhibition of MCF-7 breast cells growth in culture.

EXAMPLE 14

CELL PROLIFERATION ASSAY IN ZR-75 BREAST CANCER CELLS

[0080] ZR-75 cells are human mammary carcinoma cells that are positive for estrogen receptors. The potential activity of vitamin D₃ analogs against breast cancer was assessed from inhibition of proliferation of ZR-75 cells in culture.

[0081] ZR-75 cells were plated at 12,500 cells/well in 24-well plates and incubated at 37°C in 5% CO₂/95% air in RPMI medium containing 10% fetal bovine serum and 2 mM glutamine. Stock solutions of vitamin D₃ analogs were prepared at a concentration of 10 mM in absolute ethanol and stored at -20°C under argon. One day after plating, ZR-75 cells were refed with either control medium or medium containing varying concentrations of the vitamin D₃ analog. After a further 10 days of culture, the number of ZR-75 cells in each well was assessed from the reduction of the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide), as described by F. Denizot and R. Lang, *J. Immunological Methods*, Vol. 89:271-277 (1986). MTT was added to each well to a final concentration of 1 mg/ml and the cells were incubated for a period of three hours, after which reduced MTT was extracted using 95% ethanol and the optical density was measured at a wavelength of 570 nm.

[0082] For each vitamin D₃ analog, the IC₅₀ value was determined from a graph relating the optical density of 570 nm to the concentration used. The IC₅₀ value was defined as the concentration of the vitamin D₃ analog corresponding to half-maximal reduction in 570 nm absorbance.

Vitamin D ₃ Analogs	IC ₅₀ nM
1,25-Dihydroxy-cholecalciferol	13
21-(3-Hydroxy-3-methyl)butyl-1,25-dihydroxy-cholecalciferol	0.3

[0083] The results of the above test show that 21-(3-Hydroxy-3-methyl)butyl-1,25-dihydroxy-cholecalciferol is more

than 40 times more potent than 1,25-dihydroxy-cholecalciferol in inhibition of ZR-75 breast cells growth in culture.

EXAMPLE 15

ORAL DOSAGE FORM SOFT GELATIN CAPSULE

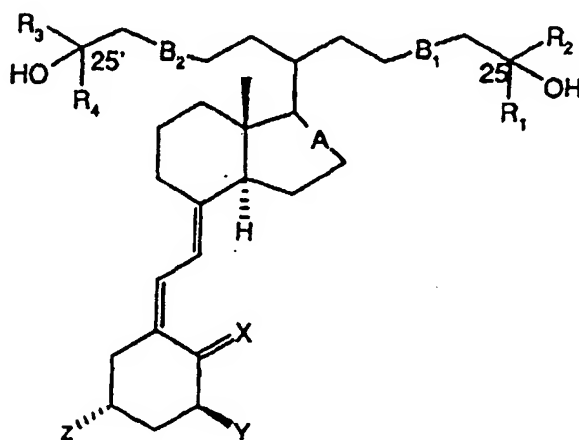
[0084] A capsule for oral administration is formulated under nitrogen in amber light from 0.01 to 25.0 mg of one of the compounds of the present invention in 150 mg of fractionated coconut oil, with 0.015 mg butylated hydroxytoluene (BHT) and 0.015 mg butylated hydroxyanisole (BHA), filled in a soft gelatin capsule.

[0085] The foregoing invention has been described in some detail by way of illustration and example, for the purposes of clarity and understanding. It will be obvious to one of ordinary skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

[0086] The patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.

Claims

1. A compound of Formula I



I

wherein:

X is H₂ or CH₂;

Y is hydrogen, hydroxy or fluorine;

Z is hydroxy,

R₁ and R₂ are a (C₁-C₄) alkyl or fluoroalkyl, or R₁ and R₂ together with C₂₅ form a (C₃-C₆) cycloalkyl or cyclofluoroalkyl;

R₃ and R₄ are a (C₁-C₄) alkyl or fluoroalkyl, or R₃ and R₄ together with C_{25'} form a (C₃-C₆) cycloalkyl or cyclofluoroalkyl;

A is a single bond or a double bond;

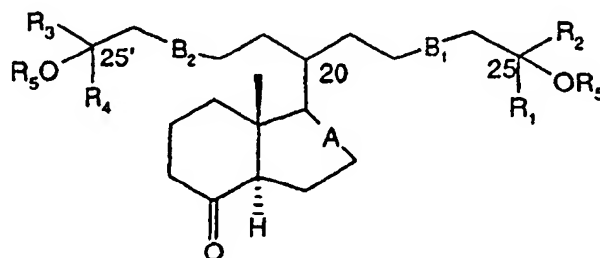
B₁ is a single bond, an E-double bond, a Z-double bond or a triple bond; and

B₂ is a single bond, an E-double bond, a Z-double bond or a triple bond.

2. The compound of Claim 1 wherein A is a single bond.

3. The compound of Claim 1 wherein Y is fluorine.

4. The compound of Claim 1 wherein Y is hydroxy.
5. The compound of Claim 1 wherein B₁ and B₂ are double bonds.
6. The compound of Claim 1 wherein B₁ and B₂ are triple bonds.
7. The compound of Claim 1 where R₁-R₄ are fluoroalkyl.
8. A prodrug of a compound of formula I as defined in Claim 1.
9. A medicament containing a compound according to any one of claims 1 to 8 and a therapeutically inert carrier.
10. Compounds according to any one of claims 1 to 8 for use as therapeutically active substances for the treatment and prevention of illnesses.
11. Compounds according to any one of claims 1 to 8 for use as therapeutically active substances for the treatment of primary and secondary hyperparathyroidism, renal osteodystrophy and autoimmune diseases.
12. Compounds according to any one of claims 1 to 8 for use as therapeutically active substances for treatment of multiple sclerosis or lupus.
13. Compounds according to any one of claims 1 to 8 for use as therapeutically active substances for the prevention and treatment of neoplastic diseases.
14. A compound of Formula II



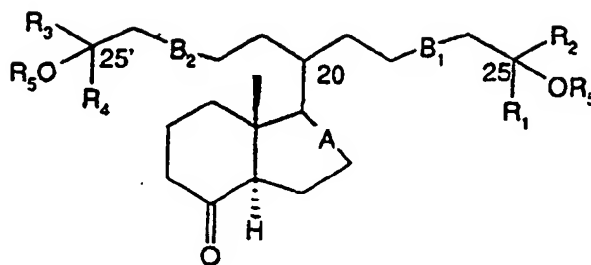
II

wherein:

- R₁ and R₂ are a (C₁-C₄) alkyl or fluoroalkyl, or R₁ and R₂ together with C₂₅ form a (C₃-C₆) cycloalkyl or cyclofluoroalkyl;
- R₃ and R₄ are a (C₁-C₄) alkyl or fluoroalkyl, or R₃ and R₄ together with C_{25'} form a (C₃-C₆) cycloalkyl or cyclofluoroalkyl;
- A is a single bond or a double bond;
- B₁ is a single bond, an E-double bond, a Z-double bond or a triple bond;
- B₂ is a single bond, an E-double bond, a Z-double bond or a triple bond; and
- R₅ is a hydroxyl protecting group.

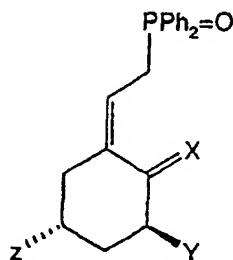
15. A method for the preparation of a compound of formula I as defined in Claim 1, comprising:

reacting a compound of Formula II



II

where R_1 , R_2 , R_3 , R_4 , A , B_1 and B_2 are as defined in claim 14, and R_5 is hydrogen or a hydroxy protecting group, with a compound of Formula III,



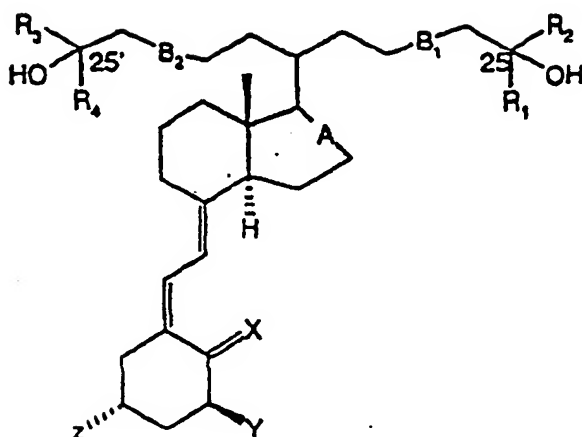
III

where X is as described above, Y is hydrogen, fluorine or a hydroxy protecting group, and Z is a hydroxy protecting group, followed by removal of the hydroxy protecting groups.

16. The use of the compounds according to any one of claims 1 to 8 for the preparation of a medicament for the treatment of primary and secondary hyperparathyroidism, renal osteodystrophy and autoimmune diseases.
17. The use of the compounds according to any one of claims 1 to 8 for the preparation of a medicament for the treatment of multiple sclerosis or lupus.
18. The use of the compounds according to any one of claims 1 to 8 for the preparation of a medicament for the prevention and treatment of neoplastic diseases.

Patentansprüche

1. Verbindung der Formel I



I

wobei:

- X H₂ oder CH₂ ist;
 Y ein Wasserstoffatom, eine Hydroxygruppe oder ein Fluoratom ist;
 Z eine Hydroxygruppe ist;
 R₁ und R₂ ein (C₁-C₄)-Alkyl- oder -Fluoralkylrest sind oder R₁ und R₂ zusammen mit C₂₅ einen (C₃-C₆)-Cycloalkyl- oder -Cyclofluoralkylrest bilden;
 R₃ und R₄ ein (C₁-C₄)-Alkyl- oder -Fluoralkylrest sind oder R₃ und R₄ zusammen mit C₂₅ einen (C₃-C₆)-Cycloalkyl- oder -Cyclofluoralkylrest bilden;
 A eine Einfachbindung oder Doppelbindung ist;
 B₁ eine Einfachbindung, eine E-Doppelbindung, eine Z-Doppelbindung oder eine Dreifachbindung ist;
 und
 B₂ eine Einfachbindung, eine E-Doppelbindung, eine Z-Doppelbindung oder eine Dreifachbindung ist.

2. Verbindung nach Anspruch 1, wobei A eine Einfachbindung ist.

3. Verbindung nach Anspruch 1, wobei Y ein Fluoratom ist.

4. Verbindung nach Anspruch 1, wobei Y eine Hydroxygruppe ist.

5. Verbindung nach Anspruch 1, wobei B₁ und B₂ Doppelbindungen sind.

6. Verbindung nach Anspruch 1, wobei B₁ und B₂ Dreifachbindungen sind.

7. Verbindung nach Anspruch 1, wobei R₁-R₄ Fluoralkylreste sind.

8. Prodrug einer Verbindung der Formel I, wie in Anspruch 1 definiert.

9. Medikament enthaltend eine Verbindung nach einem der Ansprüche 1 bis 8 und einen therapeutisch inerten Träger.

10. Verbindungen nach einem der Ansprüche 1 bis 8 zur Verwendung als therapeutische Wirkstoffe zur Behandlung und Prävention von Krankheiten.

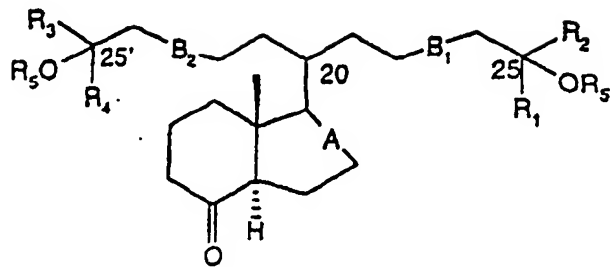
11. Verbindungen nach einem der Ansprüche 1 bis 8 zur Verwendung als therapeutische Wirkstoffe zur Behandlung von primärer und sekundärer Überfunktion der Nebenschilddrüsen, Nieren-Osteodystrophie und Autoimmunerkrankungen.

12. Verbindungen nach einem der Ansprüche 1 bis 8 zur Verwendung als therapeutische Wirkstoffe zur Behandlung von multipler Sklerose oder Lupus.

13. Verbindungen nach einem der Ansprüche 1 bis 8 zur Verwendung als therapeutische Wirkstoffe zur Prävention

und Behandlung von Tumorerleiden.

14. Verbindung der Formel II



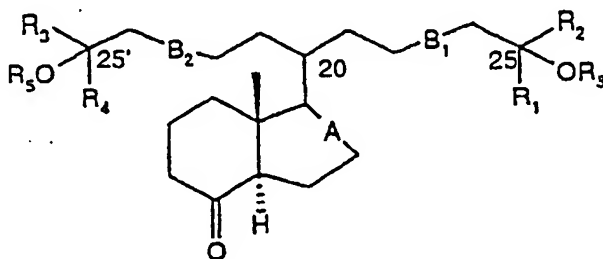
II

wobei:

- R_1 und R_2 ein (C_1-C_4) -Alkyl- oder -Fluoralkylrest sind oder R_1 und R_2 zusammen mit C_{25} einen (C_3-C_6) -Cycloalkyl- oder -Cyclofluoralkylrest bilden;
- R_3 und R_4 ein (C_1-C_4) -Alkyl- oder -Fluoralkylrest sind oder R_3 und R_4 zusammen mit $C_{25'}$ einen (C_3-C_6) -Cycloalkyl- oder -Cyclofluoralkylrest bilden;
- A eine Einfachbindung oder eine Doppelbindung ist;
- B_1 eine Einfachbindung, eine E-Doppelbindung, eine Z-Doppelbindung oder eine Dreifachbindung ist;
- B_2 eine Einfachbindung, eine E-Doppelbindung, eine Z-Doppelbindung oder eine Dreifachbindung ist; und
- R_5 eine Hydroxyl-Schutzgruppe ist.

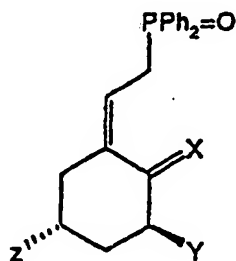
15. Verfahren zur Herstellung einer Verbindung der Formel I, wie in Anspruch 1 definiert, umfassend:

Umsetzen einer Verbindung der Formel II



II

in der R_1 , R_2 , R_3 , R_4 , A, B_1 und B_2 wie in Anspruch 14 definiert sind und R_5 ein Wasserstoffatom oder eine Hydroxy-Schutzgruppe ist, mit einer Verbindung der Formel III



III

in der X wie oben beschrieben ist, Y ein Wasserstoffatom, ein Fluoratom oder eine Hydroxy-Schutzgruppe ist,

und Z eine Hydroxy-Schutzgruppe ist, gefolgt von Entfernen der Hydroxy-Schutzgruppen.

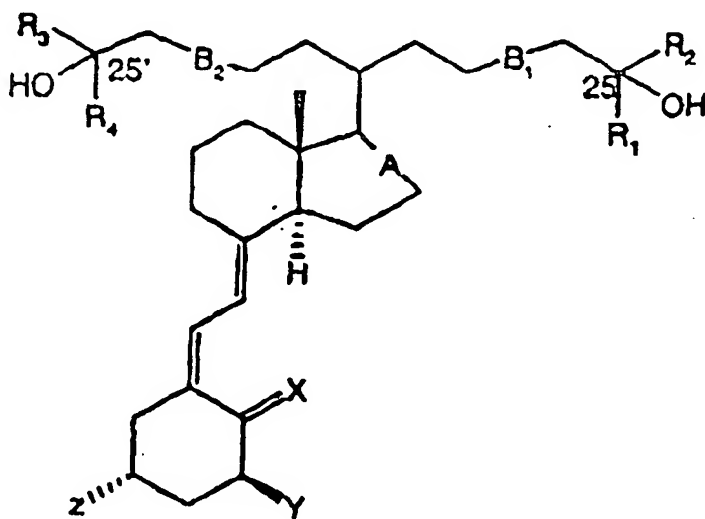
16. Verwendung der Verbindungen nach einem der Ansprüche 1 bis 8 zur Herstellung eines Medikaments zur Behandlung von primärer und sekundärer Überfunktion der Nebenschilddrüsen, Nieren-Osteodystrophie und Autoimmun-Erkrankungen.

17. Verwendung der Verbindungen nach einem der Ansprüche 1 bis 8 zur Herstellung eines Medikaments zur Behandlung von multipler Sklerose oder Lupus.

18. Verwendung der Verbindungen nach einem der Ansprüche 1 bis 8 zur Herstellung eines Medikaments zur Prävention und Behandlung von Tumorleiden.

Revendications

1. Composé de formule I



dans laquelle

X est H₂ ou CH₂ ;

Y est un atome d'hydrogène ou un groupe hydroxy ou le fluor ;

Z est un groupe hydroxy ;

R₁ et R₂ représentent chacun un groupe alkyle ou fluoralkyle en C₁-C₄, ou encore R₁ et R₂, avec C₂₅, forment un groupe cycloalkyle ou cyclofluoralkyle en C₃-C₆ ;

R₃ et R₄ représentent chacun un groupe alkyle ou fluoralkyle en C₁-C₄, ou R₃ et R₄, avec C₂₅, forment un groupe cycloalkyle ou cyclofluoralkyle en C₃-C₆ ;

A est une liaison simple ou une double liaison ;

B₁ est une liaison simple, une double liaison E, une double liaison Z ou une triple liaison ; et

B₂ est une liaison simple, une double liaison E, une double liaison Z ou une triple liaison.

2. Composé selon la revendication 1, dans lequel A est une liaison simple.

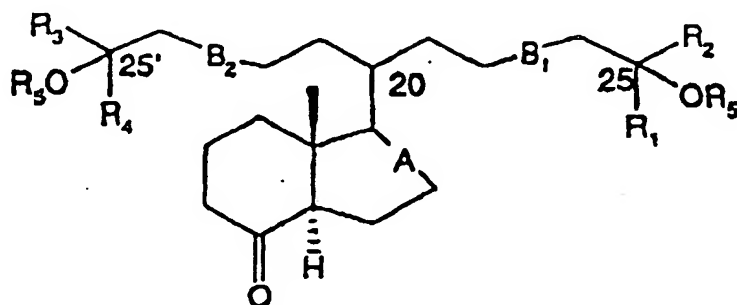
3. Composé selon la revendication 1, dans lequel Y est le fluor.

4. Composé selon la revendication 1, dans lequel Y est un groupe hydroxy.

5. Composé selon la revendication 1, dans lequel B₁ et B₂ sont des doubles liaisons.

6. Composé selon la revendication 1, dans lequel B₁ et B₂ sont des triples liaisons.

7. Composé selon la revendication 1, dans lequel R_1 - R_4 sont des groupes fluoralkyle.
8. Prodrogue d'un composé de formule I selon la revendication 1.
9. Médicament contenant un composé selon l'une quelconque des revendications 1 à 8 et un excipient inerte d'un point de vue thérapeutique.
10. Composés selon l'une quelconque des revendications 1 à 8, pour utilisation en tant que principes thérapeutiquement actifs pour le traitement et la prévention de maladies.
11. Composés selon l'une quelconque des revendications 1 à 8, pour utilisation en tant que principes thérapeutiquement actifs, pour le traitement de l'hyperparathyroïdie primaire et secondaire, de l'ostéodystrophie rénale et des maladies auto-immunes.
12. Composés selon l'une quelconque des revendications 1 à 8, pour utilisation en tant que principes thérapeutiquement actifs pour le traitement de la sclérose en plaque ou du lupus.
13. Composés selon l'une quelconque des revendications 1 à 8, pour utilisation en tant que principes thérapeutiquement actifs pour la prévention et le traitement de maladies néoplasiques.
14. Composé de formule II



II

dans laquelle

R_1 et R_2 représentent chacun un groupe alkyle ou fluoralkyle en C_1 - C_4 , ou encore R_1 et R_2 , avec C_{25} , forment un groupe cycloalkyle ou cyclofluoralkyle en C_3 - C_6 ;

R_3 et R_4 représentent chacun un groupe alkyle ou fluoralkyle en C_1 - C_4 , ou R_3 et R_4 , avec $C_{25'}$, forment un groupe cycloalkyle ou cyclofluoralkyle en C_3 - C_6 ;

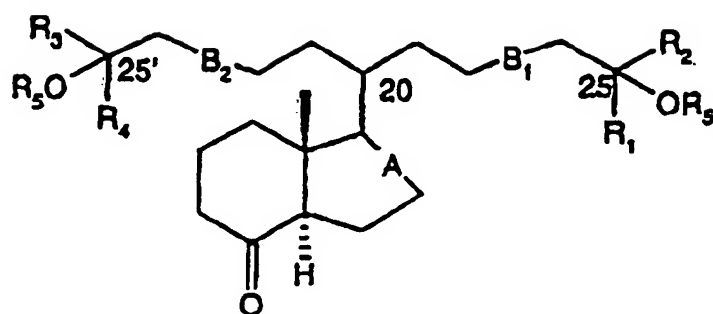
A est une liaison simple ou une double liaison ;

B_1 est une liaison simple, une double liaison E, une double liaison Z ou une triple liaison ; et

B_2 est une liaison simple, une double liaison E, une double liaison Z ou une triple liaison ; et

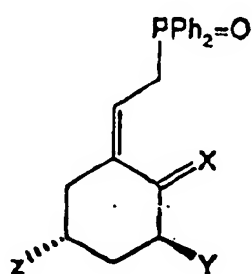
R_5 est un groupe hydroxyle-protecteur.

15. Procédé de préparation d'un composé de formule I selon la revendication 1, qui comprend la réaction d'un composé de formule II



II

dans laquelle R_1 , R_2 , R_3 , R_4 , A , B_1 et B_2 sont tels que définis dans la revendication 14 et R_5 est un atome d'hydrogène ou un groupe hydroxy-protecteur, avec un composé de formule III



III

dans laquelle X est tel que décrit ci-dessus, Y est un atome d'hydrogène ou de fluor ou un groupe hydroxy-protecteur, et Z est un groupe hydroxy-protecteur, puis l'élimination des groupes hydroxy-protecteurs.

16. Utilisation des composés selon l'une quelconque des revendications 1 à 8 pour la préparation d'un médicament destiné au traitement de l'hyperparathyroïdie pri-maire et secondaire, de l'ostéodystrophie rénale et des maladies auto-immunes.

17. Utilisation des composés selon l'une quelconque des revendications 1 à 8 pour préparer un médicament destiné au traitement de la sclérose en plaques ou du lupus.

18. Utilisation des composés selon l'une quelconque des revendications 1 à 8 pour préparer un médicament destiné à la prévention et au traitement de maladies néoplasiques.